IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Vandecruys, et al.	**Confirmation No.: 7079**
Application No.: 10/536,542	**Group Art Unit: 1618**
Filing Date: May 26, 2005	**Examiner: Jake Minh Vu**

For: Compositions Comprising a Basic Drug Compound, a Surfactant, and a Physiologically Tolerable Water Soluble Acid

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION PURSUANT TO 37 C.F.R. 1.132

I, Marcus E. Brewster, hereby declare as follows:

1. I am a Distinguished Research Fellow at Johnson & Johnson Pharmaceutical Research and Development (J&J PRD) based in Beerse, Belgium. I am currently the Head of ChemPharm Research and Early Development for Europe as well as the Chief Scientific Officer for the Chemical and Pharmaceutical division and have worked with Johnson & Johnson for 12 years. I am a Fellow of the American Association of Pharmaceutical Scientists, an former member and co-chairman of the Board of Scientific Advisors of the Controlled Release Society (2007-2009) as well as the current co-chairman of the Symposium/Workshop Committee, an Associate Editor of the Journal of Pharmaceutical Sciences (drug delivery and biopharmaceutics), a member of the Editorial Board of Die Pharmazie, a former theme editor for Advanced Drug Delivery Reviews, a member of various scientific societies and organizing boards (including the European Symposium for Controlled Drug Delivery and the International Symposium on Cyclodextrins) and the recipient of various recognitions including the J&J Excellence in Science Award (in 1998 and 2006), an Innovative Analytical Research Prize presented by FACCS (2003) and a PARC Prize for

Innovation in Pharmaceutical Analysis (2007). I have published over 245 peer-reviewed journal articles, book chapters and proceeding, have co-edited a monograph on solvents systems and their use for AAPS Press/Springer, presented over 350 meeting abstracts and was named as inventor or co-inventor on approximately 75 patents. I have also delivered more than 40 plenary lectures and 50 other invited presentations. I received my B.S. from Mercer University in 1978 and his Ph.D. from the University of Florida in 1982 in the field of Pharmaceutical Sciences. I was a visiting scientist at the Weizmann Institute of Science from 1996 to 1997.

2. I am an inventor of the above-referenced patent application. It is my understanding that the claims of the present application are directed to solid or semi-solid pharmaceutical compositions comprising a basic drug compound, vitamin E TPGS ("TPGS"), and a physiologically tolerable water-soluble acid. I also understand that methods of making these compositions are also claimed.

3. Under my direction and control, experiments were performed comparing the supersaturation effect of, for example, formulations of the present invention, on twenty-five developmental candidates having varying physicochemical properties, including molecular weight, TPSA, log P, pKa, and molecular volume. The results of these experiments were published in R. Vandecruys, et al., *Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design*, Int'l J. Pharmaceutics 342 (2007) 168-175 ("Vandecruys").

4. TPGS tended to give a higher average supersaturation as compared to Cremophor RH40 and Polysorbate 20. Vandecruys at Table 2; page 172, col. 2. As this effect was seen over a range of compounds having varying physicochemical properties, I have concluded that this effect is general. That TPGS consistently gave higher average supersaturation results as compared to Cremophor RH40 and Polysorbate 20 is surprising and unexpected in view of what was known in the art.

5. TPGS also provided better stability of the formed supersaturated solution than either Polysorbate 20 or Cremophor RH40. Vandecruys at Table 3; page 173, col. 1. As this effect was seen over a range of compounds having varying physicochemical properties, I have concluded that this effect is general. That TPGS consistently provided better stability results as compared to Cremophor RH40 and Polysorbate 20 is surprising and unexpected in view of what was known in the art.

6. Solid formulations were also prepared to assess the oral bioavailability of Compound 1 using the dog as a model. Vandecruys at page 174, col. 1. Compound 1 is a poorly soluble weak base with a water solubility of 0.002 mg%. *Id.* at 173 at col. 1. Blends of drug compound with either PEG 400, Cremophor RH40, or TPGS were prepared. *Id.* at 174 col. 1. The oral bioavailability of the drug from each formulation was determined by comparing blood levels with those obtained using an IV dose of the compound. *Id.* at page 174, col 1.

7. The results of the dog bioavailability study are shown in Table 5 of Vandecruys. As set forth therein, solubilizing the compound in PEG 400 increased the oral bioavailability of the drug compound to about 30%. Solubilizing the compound in Cremophor RH40 resulted in an oral bioavailability of about 60%. Surprisingly and unexpectedly in view of what was known in the art, using TPGS, an oral bioavailability of about 100% was achieved.

8. Also under my direction and control, experiments were performed comparing the effect on supersaturation of, for example, TPGS, Tween 20 and Cremophor RH40, for itraconazole. The results of these experiments were published in M.E. Brewster et al. *Comparative interaction of 2-hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin with itraconazole: Phase-solubility behaviour and stabilization of supersaturated drug solutions* Eur. J. Pharma. Sci. 34 (2008) 94-103 ("Brewster"). As shown in FIG. 10, formulations of itraconazole with TPGS exhibited very good levels and stability of supersaturation. See also Table 1 supporting robustness of the technique used.

9. Based on the foregoing, I concluded that solid or semi-solid compositions comprising a basic drug compound, TPGS, and a physiologically tolerable water-soluble acid exhibit extent and stability of supersaturation, and oral bioavailability profiles that are unexpected.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 15 September 09

Marcus E. Brewster

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Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design☆

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Abstract

Assessing the effect of excipients on the ability to attain and maintain supersaturation of drug-based solution may provide useful information for the design of solid formulations. Judicious selection of materials that affect either the extent or stability of supersaturating drug delivery systems may be enabling for poorly soluble drug candidates or other difficult-to-formulate compounds. The technique suggested herein is aimed at providing a screening protocol to allow preliminary assessment of these factors based on small to moderate amounts of drug substance. A series of excipients were selected that may, by various mechanisms, affect supersaturation including pharmaceutical polymers such as HMPC and PVP, surfactants such as Polysorbate 20, Cremophor RH40 and TPGS and hydrophilic cyclodextrins such as HPβCD. Using a co-solvent based method and 25 drug candidates, the data suggested, on the whole, that the surfactants and the selected cyclodextrin seemed to best augment the extent of supersaturation but had variable benefits as stabilizers, while the pharmaceutical polymers had useful effect on supersaturation stability but were less helpful in increasing the extent of supersaturation. Using these data, a group of simple solid dosage forms were prepared and tested in the dog for one of the drug candidates. Excipients that gave the best extent and stability for the formed supersaturated solution in the screening assay also gave the highest oral bioavailability in the dog.

Keywords: Supersaturation; Excipient; Cellulosic polymers; PVP; Surfactants; Cyclodextrins; Co-solvents

1. Introduction

The ability to generate formulations with relevant orally bioavailability depends on a number of factors including solubility, permeability and metabolic stability. Absorbability is related to the first two factors whose importance has been recognized in the guise of the biopharmaceutical classification system (BCS) (Dressman et al., 1998; Amidon et al., 1995). This approach bins drugs and drug candidates into four categories based on their solubility and permeability properties. The manner in which new drug candidates are selected often relies on high throughput screening techniques. These immobilized receptor techniques tend to sub-select for compounds with undrug-like attributes including high hypophilicity, poor aqueous solubility and high molecular weights (Lipinski et al., 1997; Lipinski, 2001). Retrospective analysis completed in the late 1980s suggested that many drug failures (~40%) were related in some way to the poor biopharmaceutical properties (Prentis et al., 1988). Reanalysis of drug candidate failures in 2004 indicated that some progress had been made with regard to screening out drug candidates with poor drugability elements but poor drug solubility and, by virtue of the Noyes–Whitney relationship, poor dissolution rates continue to be an important consideration in drug candi-

☆ A preliminary account of this work has been presented in poster form: Peeters J, Vandecruys R, Brewster M., 2003. The use of supersaturation studies in early pharmaceutical development. 2003 American Association of Pharmaceutical Scientists Annual Meeting and Exposition. Salt Lake City, UT, USA, 26–30 October 2003; Vandecruys R, Peeters J, Brewster M., 2006. Supersaturation studies to improve drug formulations, 2006 American Association of Pharmaceutical Scientists Annual Meeting and Exposition, San Antonio, TX, 29 October–2 November, 2006.

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date attrition (Kola and Landis, 2004). Numerous methodologies have been suggested and practically applied to improve the ability to market drug candidates whose development is limited by drug solubility, dissolution rate and by virtue of Fick's First Law, absorbability. These include the use of particle size manipulation via micronization and nanonization, the use of wetting agents, the use of complexing agents such as cyclodextrins and the preparation of high energy drug states related to polymorphic or amorphic transformations (Liu, 2000; Kim and Park, 2004; Merisko-Liversidge et al., 2003; Forster et al., 2002; Humberstone and Charman, 1997; Davis and Brewster, 2004).

One suggested path forward is the use of a spring and parachute approach wherein a technique such as solid dispersions or self-emulsifying systems allow rapid dissolution of the poorly water-soluble drug at supersaturated concentration. A formulation component which hinders nucleation or crystal growth then acts as a parachute to stabilize the metastable supersaturated systems (Guzmán et al., 2004; Gao and Morozowich, 2006; Gao et al., 2004). An example of this stratagem is the capsule-based dosage form for the antifungal compound, Sporanox (itraconazole) (Peeters et al., 2002). Itraconazole is associated with very poor formulation properties including a low aqueous solubility (estimated at ~1 ng/mL at neutral pH), a log $P > 5$ and a melting point of 167 °C. The successful preparation of an oral formulation was based on the development of a solid solution of the drug in a polymeric matrix based on HPMC. This system was prepared by a solvent method in which the drug and polymer were dissolved in a common solvent and coated onto an inert sugar sphere. In this formulation, dissolution of the water-soluble HPMC phase was associated with release of the itraconazole at concentrations above its saturation solubility. The co-dissolving HPMC acted as an inhibitor of drug nucleation and crystal growth such that supersaturated concentrations were maintained long enough for significant absorption and oral bioavailability. The maximum fraction absorbed for this formulation was ~85% and oral bioavailability as high as ~55% (Brewster et al., 2004). Other examples are available for solid dispersions prepared by melt extrusion and spray drying (Kohori et al., 1999; Crew et al., 2005; Appel et al., 2006). In addition, nanoparticulate systems can give supersaturated systems based on the greater solubility of small versus large particles (Müller et al., 1999; Lindfors et al., 2006; Wu and Nancollas, 1998).

The optimisation of system features that will allow for sustained supersaturation and by extension improved oral bioavailability is the stimulus for the current study. A number of research efforts have pointed to methods for solubilizing, that is increasing the saturation solubility, of poorly water-soluble drugs and drug candidates. Some of these have lead to marketed products (Strickley, 2004). Supersaturation approaches to enhance topical and transdermal therapies have been widely reported (Raghavan et al., 2001, 2003; Davis and Hadgraft, 1991; Moser et al., 2001a,b; Hadgraft, 1999). Less information is available on techniques to stabilize formed high energy solutions intended for oral application especially in a manner which could be useful in early formulation design and screening. Armed with such data the selection of excipient may be optimized. Herein we suggest an approach for testing excipients with regard to their ability to attain and maintain supersaturation. This approach was implemented and results on a number of early drug candidates are discussed. Finally, a case study is described where early solid formulations are generated based on excipient behaviour in the supersaturation tests.

2. Materials and methods

Research compounds were obtained from Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium. All compounds demonstrated a purity of >95%. The log P and pK_a of the drug candidates were determined experimentally while other parameters (topological polar surface area (TPSA) and molecular volume (MolVol)) were calculated using the ADME Predictor software package (Simulations Plus, Lawrenceville, CA). The following excipients were used in the studies: hydroxypropyl cellulose (HPC) 150–700 mPa s (Aqualon Belgium N.V., Doel-Beveren, Belgium), hydroxypropylmethyl cellulose (HPMC) 2910 5 mPa s (Aqualon, Hercules, Zwijndrecht, The Netherlands), Polyox NF 100k (Dow Chemical Company, Midland, MI, USA), polyvinylpyrrolidone-*co*-vinyl acetate (PVP VA)64 (BASF AG, Ludwigshafen, Germany), polyvinylpyrrolidone (PVP) K30 (BASF AG, Ludwigshafen, Germany), Cremophor RH40 (BASF, Hamolt, Belgium), Polysorbate 20 (Codibel NV, Zaventem, Belgium), α-tocopherol polyethylene glycol succinate (TPGS) (Eastman Chemical Company, Anglesey, UK), PEG4000 (Sigma–Aldrich, Bornem, Belgium). 2-Hydruxypropyl-β-cyclodextrin (HPβCD) was obtained from Roquette (Lestrem, France) and was characterized by a degree of substitution of 4.2 based on an FT-IR method (Michaud and Icart, 2001). Additionally, for the solid dosage forms the following excipients were used: lactose (DMV International, Veghel, The Netherlands), citric acid (Merck, Darmstadt, Germany), sodium lauryl sulfate (SLS) (Cognis Benelux, Hoofddorp, The Netherlands), silicone dioxide (Degussa, Frankfurt, Germany), PEG400 (Functional Chemicals, Muttenz, Switzerland), PEG20000 (Sigma–Aldrich, Bornem, Belgium), sugar spheres (Hans Werner, Tornesch, Germany), microcrystalline cellulose (MCC) (FMC, Philadelphia, USA), croscarmellose sodium (FMC, Philadelphia, USA), magnesium stearate (Univar Benelux, Brussels, Belgium). Other materials and solvents were obtained from Sigma–Aldrich (Bornem, Belgium) or Janssen Pharmaceutica (Beerse, Belgium).

2.1. Supersaturation assay

A co-solvent/solvent quench-based approach was used to generate the drug in a supersaturated state. In most cases, the drug of interest was dissolved to a concentration of 100 mg/mL in *N,N*-dimethylformamide (DMF). Dimethylacetamide (DMA) was also used in a few experiments and in isolated incidences, a 50 mg/mL solution was applied based on the limited solubility of the drug candidate. In the majority of cases the final organic content in the dissolution media ranged between 1% and 5% (v/v) although there were a few compounds where the final media contained >5% (v/v) of the organic component. A dissolution vessel

was prepared using a 20 mL glass vial with stopper. Into the vial was placed 10 mL of the media of interest (0.01N HCl, USP pH 4.5 buffer, USP pH 6.8 buffer or water) with or without 2.5% (w/v) of the excipient of interest. The vial was equilibrated at 37 °C in a water bath (Variomag Telemodule 20P) and stirred at 600 rpm using a magnetic stir bar (2 cm × 0.55 cm). The organic solution of the compound was added drop-wise using a small volume pipet into the stirring solution until a precipitate was just noticeable visually. At 5, 30, 60 and 120 min post-drug addition, a small volume of the dissolution medium was withdrawn, filtered through a 0.5 μm Millex-LCR (Millipore Corp.) filter and the concentration determined using Beer's Law with an Agilent 8543 UV spectrophotometer. The pH of the systems was measured throughout the sampling exercise (Sentron type pH meter (Titan)). In assessing the excipients, a supersaturation index was defined based on the ratio of the initial drug concentration in the excipient-based dissolution vessel as a function of that in the dissolution media that did not contain the excipient. In addition the physical stability of the solution was assessed over time with a $\Delta\%$ defined at the extent to which the drug precipitated from $C(5\ \text{min})$ to $C(120\ \text{min})$. In addition, initial supersaturation associated with addition of the drug-containing organic solvent to media without excipients was inferred based on the initial concentration values as well as the change in concentration of these systems over time.

2.2. Formulation based on the supersaturation studies

Six simple formulations were used in the animal studies including (1) a simple drug blend in a gelatin capsule, (2) a PEG400 solution, (3) a drug dispersion in HPMC E5 coated onto sugar spheres and placed in a gelatin capsule, (4) an HPβCD coprecipitate pressed into a tablet, (5) a mixture of the drug and Cremophor RH40 with additional excipients filled into a gelatin capsule, (6) a mixture of TPGS and other excipients filled into a gelatin capsule. In all cases red cap-red body capsules from Capsugel (Belgium) were used. The amount of drug in the formulations was adjusted to the weight of the dogs. In the descriptions, generic preparation methods for either 50 mg (1 unit per dog) or 25 mg (2 units per dog) nominal weight formulations are given. For the drug blend in capsule, 25 mg drug, 0.59 mg sodium lauryl sulfate, 0.59 mg silicon dioxide and 300 mg granulated lactose were mixed and filled into a size 1 capsule. The PEG400 solution contained 40 mg of Compound 1 in 1.0 mL of PEG400. The bead-based concept contained 25 mg Compound 1, sugar spheres (25–30 mesh, 236 mg), 75 mg of HPMC E5 and 13.5 mg of PEG20000 used as a seal coat. Drug and HPMC were dissolved in a mixture of 2040 mg of dichloromethane and 360 mg of ethanol. The solution was sprayed on the sugar beads using an Uni-Glatt coater with 6 in. Wurster insert (Uni-Glatt, Glatt, Germany) at a spraying rate of 15–20 g/min and an inlet temperature of 50–60 °C. In a second step, PEG20000 was dissolved in a mixture of 72.9 mg dichloromethane and 48.6 mg ethanol and sprayed on top of the first layer. The total batch size was approximately 1600 g. The coated sugar beads were filled in the hard gelatin capsules (size 0) by hand. The HPβCD-based formulation contained 25 mg of Compound 1, 75 mg of HPβCD, 14 mg of citric acid monohydrate, 243 mg of granulated lactose, 60 mg of microcrystalline cellulose, 30 mg of croscarmellose sodium and 2.3 mg of magnesium stearate. The drug, cyclodextrin and citric acid were mixed in acetone and ethanol after which the solvent was removed. The co-precipitate was blended with the other listed excipients and hand pressed into a tablet. For the concept based on Cremophor RH40, the drug (50 mg), the surfactant (250 mg) and citric acid monohydrate (500 mg) were dissolved in ethanol after which the solvent was removed and the residue manually loaded into a size 00 capsule. The last concept was similarly prepared by blending the drug (50 mg), TPGS (250 mg) and 25 mg of HPMC E5 in ethanol, removing the solvent and then loading the residue into a size 0 capsule. The latter two concepts are based on a glass thermoplastic approach (Brewster et al., 2003). The dissolution of the capsules was assessed using a USP II apparatus using 600 mL of 0.01N HCl medium equilibrated at 37 °C. The paddle stirred at 75 rpm and data are given as the average of two units.

2.3. Bioavailability in the dog

All animal studies were completed according to relevant Belgium Law (18 October 1991) as approved by the European Convention on the protection of vertebrates that are used for experimental and other scientific purposes, Annex A and B as drawn up in Strasbourg (18 March 1986) and the Royal Decree of 14 November 1993 covering the protection of laboratory animals. All experiments were planned and completed under the auspices of the Institutional Animal Care and Use Committee (IACUC). Male Beagle dogs were obtained from Harlan, Germany and maintained in the resident Janssen Pharmaceutica dog colony. Their body weight ranged from 11 to 13.4 kg at the start of the study. Dogs were healthy as judged from routine blood examinations (haematological and biochemical parameters) and certified by the attending veterinarian. All animals were observed prior to, at the time of dosing and up to 24 h post-dosing. Dogs had free access to water and food through the experimental period. Fed animals were dosed using a cross-over design with a washout period of 14 days. Solid formulations were dosed orally. Oral solutions were administered by gavage using a stomach tube. The IV arm of the study was completed using a dose of 1.25 mg/kg and a vehicle containing 75:25 PEG:water. Blood samples were obtained from the jugular vein (3 mL collected onto EDTA) just prior to dosing and at the following time points thereafter: 0.5, 1, 2, 4, 6, 8, 24, 32, 48, 72 and 96 h. Blood samples were centrifuged at room temperature at 1900 × g for 10 min to separate the plasma. The plasma was transferred to a second test tube and frozen within 2 h of sampling at −20 °C. Samples were analysed using a validated LC–MS/MS method. Plasma samples (0.1 mL) were subjected to solid phase extraction (Bond Elut solid phase columns, 130 mg, SPE (Varian Corp)). Columns were conditioned with 3 mL methanol, 3 mL water and 1 mL acetic acid (1 M). Plasma was applied to column and washed with subsequent aliquots of water, acetic acid and methanol. The compound was eluted from the column with 3 mL methanol/ammonium

hydroxide (25% aqueous) at a ratio of 98:2. The extract was evaporated to dryness and reconstituted with 150 μL of a 50:50 mixture of ammonium formate (0.01 M) and methanol. Twenty microliters of the sample was then applied to a 10 cm × 4.6 mm i.d. reversed phase HPLC column (3 μm Hypersil C18 BDS) and eluted with a mobile phase containing 40% 0.01 M ammonium formate and 60% methanol flowing at 0.8 mL/min (before splitting). LC–MS/MS analysis was completed using an API-3000 system (Applied Biosystems) which was dedicated to the HPLC (Agilent). The lower limit of quantification was 1 ng/mL. Individual plasma concentration–time profiles were subjected to a non-compartmental pharmacokinetic analysis using WinNonlin software (Pharsight Corp., Mountain View, CA, USA). The area under the plasma curve (AUC) from time 0 to 24 or 96 h was calculated using the linear up/down trapezoid rule. Oral bioavailability was reported as the ratio of $(AUC_{oral}/AUC_{iv}) \times 100$.

3. Results and discussions

Excipients which stabilize formed supersaturated solutions may be important additives to solid formulations. A method was developed to screen for such excipients based on a co-solvent approach. Twenty-five developmental candidates were screened using this assay. Physicochemical properties including molecular weight, TPSA, log *P*, pK_a and molecular volume for the screened materials are presented in Table 1. The compounds cover a reasonable chemical space with MW ranges between 327 and 721, TPSAs between 46 and 198 Å^2, log *P*s between 1.76 and >5 and molecular volumes between 273 and 602 Å^3. The effect of various excipients on the extent of supersaturation is given in Table 2 while the stability of the formed supersaturated solution in the presence of the specified excipient is given in Table 3. The excipients were selected based on a variety of potential mechanisms which may impact the ability of the material to nucleate or to arrest crystal growth. The exact mechanisms associated with nucleation and crystal growth are not well-described. Crystal growth is believed to take place in three steps (Macie and Grant, 1986; Rodriguez-Hornedo and Murphy, 1999; Raghavan et al., 2001):

- diffusion of the molecule from the bulk media to the solid crystal interface;
- the adsorbed molecule, through a surface reaction, becomes part of the crystal lattice and the heat of crystallization is released;
- the heat of crystallization is conducted to the bulk media.

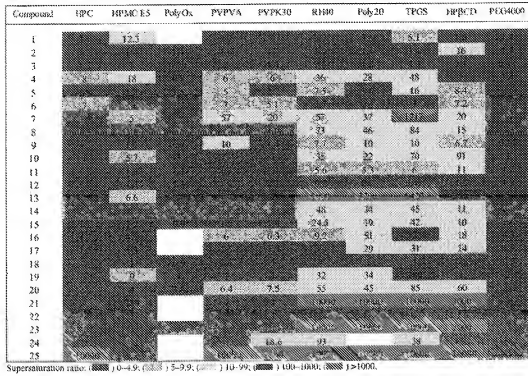
Materials that may inhibit nucleation or crystal growth have been reported. These materials have several potential actions including:

- altering bulk properties such as surface tension or saturation solubility;
- changing the adsorption layer at the crystal-medium interface;
- selectively adsorbing to the crystal interface thereby blocking crystal growth;
- being adsorbed into growth layers and thereby disrupting growth layers across the surface;
- adsorbing into surface imperfections causing rough surfaces to become flat;

Table 1
Compound properties, dissolution media and initial and final concentrations in the dissolution media

Compound	MW	TPSA (Å^2)	log *P*	pK_a	MolVol (Å^3)	Media	*C*(5 min) (mg%)	*C*(120 min) mg%	Δ%
1	366.4	97	4.8	5.6	352	0.01N HCl	18	16	−11
2	373.4	80	1.58	1.86	306	0.01N HCl	14	14	0
3	486.5	107	4.97	4.9	392	0.01N HCl	41	8	−80
4	453.5	150	3.9	3.53	385	0.01N HCl	0.05	0.03	−40
5	538.7	108	3.9	Multiple	559	pH 6.8	5.5	0.21	−96
6			1.76	3.68		0.01N HCl	34	10	−71
7	547.4	109	4.49	6	375	pH 6.8	0.3	<0.001	–
8	441.4	131	3.05	10.12	340	0.01N HCl	7.3	1.8	−75
9	353.4	82	2.13	2.2; 8.9	308	0.01N HCl	92	9	−90
10	413.5	47	2.72	8.18	322	0.01N HCl	0.23	<0.001	–
11	327.5	49	4.27	–	336	Water	38	38	0
12	573.7	198	3.29	2.2	490	pH 6.8	71	4	−94
13	504.5	101	5.03	5.72	410	0.01N HCl	<0.05	<0.05	–
14	686.8	47	4.43	7.2; 9.1	602	pH 6.8	9	4	−56
15	573.7	108	3.29	2.2	490	0.01N HCl	17	18	6
16	720.9	108	4.94	Multiple	686	0.01N HCl	3	7	130
17	676.7	130	4.88	7.5; 3.6	585	pH 4.5	13	3	−77
18	427.5	97	2.4	8.26	303	0.01N HCl	397	–	–
19	344.4	94	2.9	–	306	0.01N HCl	<0.01	<0.01	–
20	371.4	58	2.9	2.1; 8.5	343	0.01N HCl	0.4	0.4	0
21	499.4	50	5.11	2.8	424	0.01N HCl	<0.001	<0.001	–
22	555.5	46	5.14	9.1	466	0.01N HCl	351	364	4
23	572.6	59	>5	6.3	487	pH 6.8	<0.001	<0.001	–
24	394.4	154	2.15	3; 9	273	0.01N HCl	5	5	0
25	639.8	84	>5	8.5	509	0.01N HCl	<0.001	<0.001	–

Table 2
The extent of supersaturation observed for various compounds in the presence of 2.5% (w/v) of various excipients



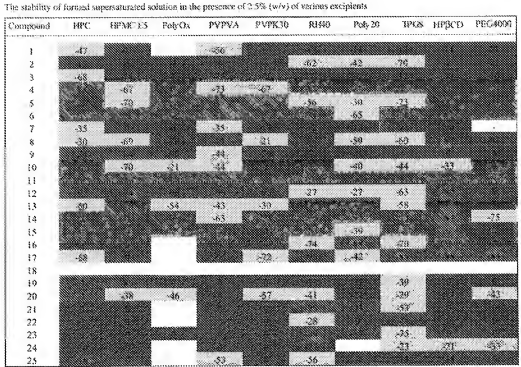
Supersaturation ratio: ( ) 0–4.9; ( ) 5–9.9; ( ) 10–99; ( ) 100–1000; ( ) >1000.

- altering the surface energy of the crystal face which may change the level of solvation.

Rheological polymers such as HPMC and PVP are thought to interact through a number of mechanisms including adsorbing to the crystal (via hydrogen bonding) and collecting at the growing crystal-bulk media interface and thereby providing diffusion resistance (Raghavan et al., 2001). Some reports also suggest that these polymers can form complexes with the drug of interest, increase their saturation solubility and therefore reduce the extent of supersaturation (Rodríguez-Hornedo and Murphy, 1999; Strickley, 2004). Surfactants can solubilize materials via micelle formation but can also alter the surface tension at the crystal-medium interface (Constantinides et al., 2006; Rangel-Yagui et al., 2005). Cyclodextrins can solubilize material through the formation of dynamic inclusion complexes (Loftsson et al., 2004; Loftsson and Brewster, 1996; Thompson, 1997; Rajewski and Stella, 1996). Additional data suggest that cyclodextrins can also inhibit nucleation and crystal growth through non-complex based mechanisms which may be similar to those associated with the pharmaceutical polymers described above (Brewster et al., 2006; Torres-Labandeira et al., 1990; Uekama et al., 1992). PEG4000 or Polyox may affect supersaturated solutions through various mechanisms (Li et al., 2006; Urbanetz and Lippold, 2005).

The cellulosic, PEO/PEG and PVP-based polymers had variable effects on the formed supersaturated solution. HPC gave generally poor results with two compounds (Compounds 4 and 6) giving solubility increases in the 5–10-fold range and one compound (Compound 25) providing a significant increase over the baseline. In this case, the absolute value of the drug concentration in the supersaturated solution was small (11 mg%). HMPC was somewhat more conductive for the formation of supersaturated solutions with 7 hits, 4 in the range of 5–10-fold (Compounds 7, 10, 13 and 19), 2 in the range of 10–20-fold (Compounds 1 and 4) and in one instance the excipient provided for a 280-fold increase in concentration (Compound 21). Again in this case, the absolute values of the formed solution was low (0.5 mg%) and the enhancement was related to the very poor solubility of the compound in the dissolution media without excipients. Polyox and, not unexpectedly, PEG4000 were the worst excipients in forming supersaturated solutions in that there was not a single example of a concentration increase above three-fold or so. The surfactants, as a class, better supported the formation of supersaturated solutions. There were 17-hits in the case of both Cremophor RH40 and Polysorbate 20 and 20-hits for TPGS. TPGS also tended to give a higher average supersaturation compared to the other two materials. Compounds that gave the highest absolute values in the supersaturation test for TPGS included Compound 9, 885 mg%, Compound 17, 710 mg% and Compound 8, 614 mg%. HPβCD also provided for useful values with 18 of the 25 compounds demonstrating supersaturation ratios >5. The highest ratios were obtained for Compounds 13, 21, 23 and 25 while the highest absolute values were available

Table 3
The stability of formed supersaturated solution in the presence of 2.5% (w/v) of various excipients



for Compound 9, 625 mg%, Compound 25, 421 mg%, Compound 11, 414 mg% and Compound 6, 244 mg%. These data are in contrast to other reports which suggest that cyclodextrins were poor excipients in supporting supersaturation and, in some cases, accelerated crystallization (Dias et al., 2003; Ma et al., 1996; Iervolino et al., 2000) Simple correlation between supersaturation ratios and individual physicochemical parameters given in Table 1 was poor suggesting complex interactions between the excipients and the drug candidates.

An indication of the stability of the formed supersaturated systems is provided in Table 3. Taken in isolation, the data suggest that HPC provides stable solution in 12 out of 25 instances while there were 14 hits for HMPC. Polyox and PEG4000 provided stable solutions in 8 and 15 cases, respectively. The surfactants could be stratified based on stability with TPGS > Polysorbate 20 > Cremophor RH40. These data cannot, however, be used in and of themselves and should be combined with the information in Table 2. That is information on both the extent and stability of the supersaturated solutions is needed to select appropriate excipients for subsequent development. To this point, an example may be illustrative.

Compound 1 is a poorly soluble weak base with a water solubility of 0.002 mg% and solubilities in 0.01N HCl of 1.9 mg% and 0.1N HCl of 1.3 mg%. When a DMA solution is added to 0.01N HCl, a supersaturated solution is generated at 18 mg% (i.e., nine-fold higher than the equilibrium solubility in 0.01N HCl) which is maintained through 120 min with an 11% decrease in concentration. Table 4 gives the time versus concentration data obtained in the supersaturation assessments. In evaluating the first line of Tables 2 and 3, the excipient that gave the highest extent of supersaturation was HPMC which gave a 12-fold increase over the solubility generated in the medium without excipient and greater than 100-fold increase over the equilibrium solubility in 0.01N HCl and TPGS where a five-fold improvement over the medium and an almost 50-fold increase over the

Table 4
Concentration (in mg%) of Compound 1 over time in a dissolution media (0.01N HCl) containing 2.5% (w/v) of various excipients

Time (min)	0.01N HCl	HPC	HPMC E5	PVPVA	PVPK30
5	18	38	222	41	16
30	16	24	27	22	14
60	16	20	21	20	13
120	16	20	20	18	13
Final pH	1.99	2.30	2.38	2.29	2.30
Final % organic solvent	3.4	2.9	2.4	3.1	2.9
Time (min)	RH40	Poly20	TPGS	HPβCD	PEG4000
5	57	65	92	28	25
30	52	58	84	25	22
60	51	57	76	24	21
120	50	56	75	24	20
Final pH	2.11	2.10	2.36	2.0	2.29
Final % organic solvent	3.7	3.7	5.3	2.7	2.6

Table 5
Biopharmaceutical properties and oral bioavailability of various early solids concepts for Compound 1

Formulation concept	Dissolution[a] (%)	C_{max} (ng/mL)	t_{max} (h)	AUC (ng h/mL)	Oral Bio (%)
Drug-in-capsule	47	21	4	308	1.3
PEG400 solution	--	341	4	8,359	31
HPMC E5 coated bead	94	449	2	11,778	58
HPβCD-based tablet	88	761	6	18,129	58
RH40-based capsule	78	816	3	16,961	58
TPGS-based capsule	98	1145	5	31,008	106

[a] Dissolution at 30 min (see Section 2).

equilibrium solubility was observed. In assessing stability, the worst excipient was HMPC with compound levels falling 90% over the 120 min experiment time course. The concentration of drug obtained using TPGS as the excipients were sustained with an 18% loss between the 5 and 120 min time point.

Solid formulations were prepared to optimize the oral bioavailability of Compound 1 using the dog as a model. The formulations that were prepared included a simple drug blend in capsule, a PEG400 solution, a capsule containing beads onto which was deposited a solid dispersion of the compound in HPMC (Verreck et al., 2004), a tablet based on a co-precipitate of the drug with HPβCD, the compound dissolved in Cremophor RH40 and filled into a gelatin capsule and the drug formulated with TPGS and filled into a gelatin capsule. The amount of drug was adjusted such that dogs received 5 mg/kg of the compound. The oral bioavailability of the drug from each concept was determined by comparing blood levels with those obtained using an IV dose of the compound. The data are presented in Table 5. The bioavailability of the compound out of a simple blend was poor consistent with its high crystallinity (m.p. = 256 °C), low solubility, high log *P* and weakly basic nature. Solubilizing the compound in PEG400 increases the bioavailability to 30% but the poor effect of PEG4000 on supersaturation suggests further improvements might be possible. To that end, a concept based on HPMC E5 was assessed. The oral bioavailability of this concept was slightly better than that associated with the PEG400 solution. That is while HPMC may give a good effect on supersaturation extent, the compound so formulated may rapidly precipitate negating any advantage. The cyclodextrin tablet increased the bioavailability in the dog to almost 60% meaning the modest solubility increase (1.6-fold versus medium without excipient and 15-fold versus equilibrium solubility in 0.01N HCl) together with the stabilizing effect of the excipient combined to give a useful solid formulation. Similar results were obtained with Cremophor RH40 which was comparable to the cyclodextrin with respect to both the extent and stability offered to the formed supersaturated solutions. The solid concept with the best performance was based on TPGS where a bioavailability of 100% was achieved. In this case, it is possible that the combination of the increased extent of supersaturation as well as the resulting stability was optimal with regard to the compound assessed. In this limited sample size, it may be concluded that while generating supersaturated solutions at increased concentrations is important, the stability of the formed systems may be the main driver in this excipient-based formulation approach.

4. Conclusions

The data collected herein suggest that a knowledge of how excipients interact with developmental drug candidates may be useful in designing oral solid dosage forms. Two factors seem to be important including the extent to which the excipient can increase supersaturation and the stability of the formed systems. Such data is useful in preparing systems based on solid dispersions, nanoparticles or the amorphous form of the drug. In all of these cases, the high energy form of the drug may dissolve at supersaturated concentration which may, from both a thermodynamic as well as a concentration point of view, be enabling for difficult-to-formulate materials. In the example cited, information on supersaturating excipients was useful in designing simple solids. The approach described is a useful place to start to generate possible excipients with added value in a solid formulation. It is just a start however. Follow-up information should include assessing synergies between different excipients or excipient types. In addition, it may be possible to select a polymeric carrier which also has good supersaturating properties as in the case of HPMC and itraconazole (Verreck et al., 2003; Six et al., 2003) or HPβCD and itraconazole (Rambali et al., 2003).

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Comparative interaction of 2-hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin with itraconazole: Phase-solubility behavior and stabilization of supersaturated drug solutions

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ABSTRACT

Cyclodextrins can increase the apparent solubility and dissolution rate of poorly water-soluble drug candidates improving their biopharmaceutical performance. The current data assess the ability of hydrophilic cyclodextrins to solubilize compounds via stabilization of supersaturated drug solutions presumably by inhibition of nucleation and arresting crystal growth. To these points, the effects of 2-hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether-β-cyclodextrin (SBEβCD) on equilibrium solubility was assessed via phase-solubility analysis as were the interactions of these excipients on drug solubility under conditions favoring supersaturation. Phase-solubility analysis indicated that different profiles were generated as a function of the cyclodextrin examined and the pH of the complexing medium. When kinetic solubility measurements were completed, the cyclodextrins were found to stabilize concentrations of itraconazole significantly in excess of their equilibrium solubility when supersaturated solutions were formed using the co-solvent/solvent quench approach. These solutions were stable over 240 min falling in concentration at the 24 h time point of the experiment unlike those formed using surfactants and other polymers which demonstrated a rapid decrease in concentration over time. These data suggest that hydrophilic cyclodextrins might be useful formulation adjuncts in supersaturating drug delivery systems.

1. Introduction

Cyclodextrins are functional excipients that have gained widespread use and attention because of their ability to solubilize, and in some instances stabilize, poorly water-soluble drug candidates enabling both oral and parenteral formulation (Fig. 1) (Davis and Brewster, 2004; Loftsson et al., 2004a; Loftsson and Brewster, 1996; Thompson, 1997; Rajewski and

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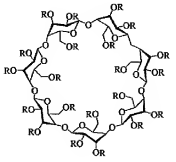


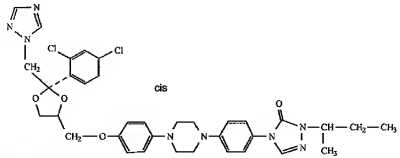
Cyclodextrin	R = H or
β-Cyclodextrin	-H
2-Hydroxypropyl-β-cyclodextrin	$-CH_2CHOHCH_3$
Sulfobutylether-β-cyclodextrin sodium salt	$-(CH_2)_4SO_3^-Na^+$

Fig. 1 – Chemical structure of β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether-β-cyclodextrin (SBEβCD).

Stella, 1996). In addition, these materials can convert oils and viscous liquids into free flowing powders and can suppress vapor pressure and therefore can reduce unaesthetic smells and tastes (Brewster et al., 1989). In the case of solubilization and for many of the other property improvements observed, the mechanism suggested is the formation of dynamic inclusion complexes in solution between the cyclodextrin and the compound of interest (Szejtli and Osa, 1996; Szejtli, 1988). This interaction occurs because of the specialized architecture of the cyclodextrin molecule wherein the material takes the form of a truncated cone or torus with the primary hydroxyl functions oriented to the narrower end of the torus and the secondary hydroxyl function oriented towards the wider end (Loftsson and Brewster, 1996; Brewster et al., 1989). This makes the exterior of the molecule relatively hydrophilic while the cavity interior is relatively hydrophobic so that the starch derivative has some degree of water solubility. At the same time, the interior lipophilic cavity allows appropriately sized lipophiles to interact resulting in solubilization.

While complexation describes many of the pharmaceutically and biopharmaceutically relevant attributes of cyclodextrins, some observations suggest that other factors are also involved. Loftsson et al. (2002, 2004b) found, for example, that in addition to complexation, other non-complex related phenomena can contribute to drug solubilization including the formation of aggregates in solution as well as surface active properties of the drug–cyclodextrin system. In the model developed, solubilization of the drug is a function of both complexation and non-complex-based association and was based on discrepancies between ideal and observed phase-solubility behavior.

The current work looks to explain several observations that seem to be in contradiction to pure complex-driven effect of cyclodextrins using itraconazole as a probe molecule. Itraconazole is a broad-spectrum antifungal agent which is marketed in an oral and i.v. solution containing HPβCD (Sporanox®) in the US, Europe and the Far East (Davis and Brewster, 2004). Certain hydrophilic cyclodextrins appear to have differential effects, both qualitatively and quantitatively,



Itraconazole

Fig. 2 – Chemical structure of itraconazole.

on the solubilization profile on itraconazole (Fig. 2). Brewster et al. (2007) reported that solubilization of crystalline itraconazole in 20% w/v HPβCD in an acidic medium was associated with a day 1 solubility value of 350 μg/mL which increased slowly over time to give a day 28 value of 500 μg/mL. Conversely, amorphous itraconazole generated 11.6 mg/mL at a day 1 measurement which slowly decreased to 2 mg/mL at day 28. One explanation for these observations is the possible effect of HPβCD as a stabilizer of the formed supersaturated system via nucleation and crystal growth inhibition. The ability of cyclodextrin to act in this capacity has been suggested by several authors (Loftsson et al., 2004c; Pedersen, 1997; Uekama et al., 1992; Ma et al., 1996; Xiang and Anderson, 2002; Torres-Labandeira et al., 1991; Dias et al., 2003; Iervolino et al., 2000, 2001).

2. Materials and methods

2.1. Materials

Crystalline and amorphous itraconazole was obtained from Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium. The following excipients were used in the studies: hydroxypropyl methylcellulose (HPMC) 2910 5 mPa s (Aqualon, Hercules, Zwijndrecht, the Netherlands), Cremophor RH40 (BASF, Hamoir, Belgium), Polysorbate 20 (Codibel NV, Zaventem, Belgium), α-tocopheryl succinate esterified to polyethylene glycol 1000 (TPGS) (Eastman Chemical Company, Anglesey, UK). HPβCD was obtained from Roquette (Lestrem, France) and was characterized by a degree of substitution of 4.2 based on an FT-IR method (Michaud and Icart, 2001). SBEβCD was obtained from CyDex Pharmaceuticals, Inc. (Lenexa, KS, USA). Phosphate–citrate buffers were used for pHs 4 and 7 media. The pH 7 buffer contained a mixture of a 0.1 M citric acid solution and 0.2 M disodium hydrogen phosphate solution (17.65:82.35 v/v) while the pH 4 buffer contained a 61.45:38.55 v/v mixture of the citrate and phosphate components.

2.2. Phase-solubility analysis

Complexes were prepared by sonicating an excess of itraconazole in various concentrations of aqueous HPβCD or SBEβCD ranging from 0 to 20% w/v prepared in several systems (water, 0.1N HCl, 0.01N HCl and pHs 4 and 7 citrate–phosphate buffer) (Peeters et al., 2002). After 10 min of sonication, the suspensions were equilibrated for 3 days at which time, a small volume of the supernatant was withdrawn and filtered through a 0.45 μm polyvinylidene difluoride (PVDF) membrane (Nihon Millipore). Samples were then diluted with 0.01N HCl and analyzed by UV (at 254 nm) using a Hewlett Packard 8451B diode array spectrophotometer. Experiments were completed in triplicate. The solubility and chemical stability of itraconazole was also assessed by HPLC in certain experiments. The systems configuration included a Varian LC 9010 solvent pump, a Varian 9096 autosampler fitted with a 10 μL sample loop and a Varian 9065 Polychrom diode array detector (for itraconazole, λ = 268 nm) dedicated to a Compac Descpro PC. Samples were eluted on an RP 18 Hypersil ODS column (10 cm × 4.0 mm i.d., 3 μm particle size) using a flow rate of 1.6 mL/min and a mobile phase composition of ammonium acetate (0.5%):methanol:acetonitrile (35:14:51). Dibutyl phthalate served as the internal standard. Under these conditions, the retention time of itraconazole was 4.0 min and for the internal standard, 4.62 min. To avoid adsorption of the drug on the filters and transferring devices, the membranes and syringes were treated using a saturated solution of itraconazole in 0.01N HCl. The syringes and filters were flushed with 3 mL of the itraconazole solution four times prior to analytical sample handling. Itraconazole is chemically stable under the conditions of the analysis. The stoichiometry of various phase-solubility isotherms is estimated by curve-fitting to various polynomials to generate trial values as presented in the appropriate figures.

2.3. Solubilization by surfactants

An excess of itraconazole was added to water adjusted to pH 5 containing various percentages of TPGS (2.5, 5, 10, 15, 20% w/v) and the sample were equilibrated for 10 days. Samples were filtered through a 0.45 μm PVDF membrane and analysed by HPLC as described in the previous section.

2.4. Supersaturation testing

Several types of experiments were completed all of which were based on the co-solvent method (Raghavan et al., 2001, 2003; Davis and Hadgraft, 1991). In this approach, a drug in a solvent providing good solubility is added to a second solvent in which the drug has little or no solubility. This creates a situation where highly supersaturated solutions can be almost instantaneously formed and is similar, in form, to the techniques used to assess kinetic solubility. In a preliminary assessment, a solution of itraconazole was prepared in dimethylformamide (DMF) (50 mg/mL). The solution was then added dropwise into the dissolution media until a precipitate formed. Dissolution was completed according to the USP (apparatus II) using 300 mL of 0.1N HCl equilibrated at 37 °C with or without 500 mg HPMC. The solution is stirred at 150 rpm. At 5, 30, 60 and 120 min after addition of the solution, a small volume of the suspension is removed from the vial, filtered and the concentration determined by UV as described above. For the second type of experiment, a screening assay was developed. In this instance the 50 mg/mL solution of itraconazole in DMF was added in small aliquots over a prescribed time period into 10 mL of dissolution solvent, in this case 0.01N HCl with (2.5% w/v) and without the excipient to be tested. Just at the point when a precipitate was observable, the addition of the drug solution was stopped and at 5, 30, 60 and 120 min thereafter the dissolution bath was sampled, the sample filtered and assayed spectrophotometrically to provide the concentration of itraconazole remaining in solution as described in the section above. The third group of experiments, which extended the characterization of TPGS, HPβCD and SBEβCD-based systems, were similarly completed however they used an $n = 3$ and provided additional time points at 180, 240 and 1440 min to better characterize the longevity of the supersaturated system.

3. Results

3.1. Phase-solubility analysis

Phase-solubility analysis of itraconazole in the presence of the two cyclodextrins was completed in turn (Loftsson et al., 2002, 2005; Peeters et al., 2002; Higuchi and Connons, 1965; Brewster and Loftsson, 1999, 2007). The total solubility (S_{Total}) of a drug as a function of cyclodextrin concentration is given by:

$$S_{Total} = S_0 + m[D_m CD]$$

where S_0 is the drug concentration in the absence of cyclodextrin and m refers to the stoichiometry of the drug-cyclodextrin complex. If one cyclodextrin molecule interacts with one drug molecule, complex characteristic can be assessed in part using phase-solubility analysis such that the slope of the phase-solubility relationship can be given by:

$$\text{Slope} = \frac{S_0 K_{1:1}}{S_0 K_{1:1} + 1}$$

where $K_{1:1}$ is the stability constant for the complex. Higher order complexes require other techniques for the calculation of their stability constants. Historically, higher order complexation (that is the interaction of one drug with a number of cyclodextrin units) has been suggested by a positive deviation from linearity for the phase-solubility relationship and deconvolution of the curve can give numerical solutions for the stability constants (Higuchi and Connons, 1965; Brewster and Loftsson, 1999). For a second-order relationship, fitting the data to a quadratic model is appropriate:

$$S_{Total} = S_0 + K_{1:1} S_0[CD] + K_{1:1} K_{1:2} S_0[CD]^2$$

however this model assumes that the total CD concentration is estimated by the free (i.e., uncomplexed) CD concentration. If this assumption does not hold, the paradigm suggested by Higuchi and Kristiansen should be used (Peeters et al., 2002; Higuchi and Kristiansen, 1970). For 1:3 interactions, a third-order curve-fitting is appropriate. To account for the concentration of bound cyclodextrin in these cubic relationships, Peeters et al. (2002) suggested a non-linear optimization technique based on an iterative Nelder-Mead approach.

The relationships between the two solubilizers and drug in water (pH ~ 6.5), pHs 7 and 4 buffer, 0.01N HCl (pH ~ 2) and 0.1N HCl (pH ~ 1.5) are given in Figs. 3–7. In water, the isotherm gives an A_P-type profile which can suggest the formation of higher order complexes at higher cyclodextrin concentrations. In addition, the fact that it is difficult to identify an initial linear segment of the isotherm may also suggest non-inclusion complex related phenomena including cyclodextrin aggregation or surfactant-based effects (Loftsson et al., 2004b). In any case, there is a significant effect of HPβCD on solubility of itraconazole such that the drug concentration increases from ~1 ng/mL in the absence of cyclodextrin to 4 μg/mL at 2.5% w/v HPβCD and 330 μg/mL at 20% w/v HPβCD (Fig. 3). For SBEβCD an A_P-type profile is also observed. Various groups have reported that this strongly suggests the intervention of non-inclusion complex effects since coloumbic repulsion discourages higher order complexation based on charge interaction for this negatively charged cyclodextrin (Thompson, 1997; Loftsson et al., 2002; Okimoto et al., 1996; Zia et al., 1997). The apparent equilibrium solubility of itraconazole in 2.5% SBEβCD was measured at 2 μg/mL while that in 10% w/v cyclodextrin the value was 14 μg/mL. In a buffered media at pH 7, the data was qualitatively similar (Fig. 4). At pH 4, HPβCD continued to provide for better relative solubility as a function of cyclodextrin concentration although the effect was lower (21-fold increase for HPβCD compared to SBEβCD at pH 7 and 8-fold at pH 4 at 10% w/v cyclodextrin) (Fig. 5). Curve-fitting suggested that complexation for itraconazole with both HPβCD and SBEβCD at pHs 7 and 4 was best using a cubic equation consistent with published work on itracona-

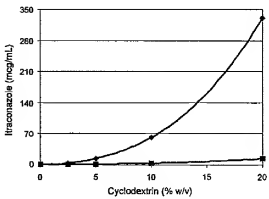


Fig. 3 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in water ($n = 3$). The calculated lines represent fitting of the data to a third-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HPβCD, $r^2 > 0.999$ and for SBEβCD, $r^2 > 0.999$. Note that error bars are within the symbol.

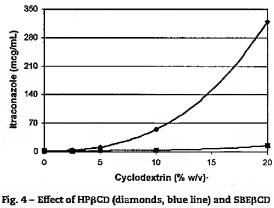


Fig. 4 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in pH 7 phosphate-citrate buffer ($n = 3$). The calculated lines represent fitting of the data to a third-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HPβCD, $r^2 > 0.999$ and for SBEβCD, $r^2 = 0.998$. Note that error bars are within the symbol.

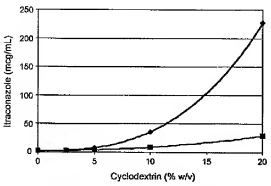


Fig. 5 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in pH 4 phosphate–citrate buffer ($n=3$). The calculated lines represent fitting of the data to a third-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HPβCD, $r^2 > 0.999$ and for SBEβCD, $r^2 > 0.999$. Note that error bars are within the symbol.

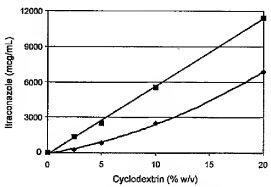


Fig. 7 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in 0.1N HCl ($n=3$). The calculated lines represent fitting of the data to a second-order model in the case of HPβCD ($r^2 > 0.999$) and a first-order model in the case of SBEβCD ($r^2 > 0.999$) which was optimal in this case based on goodness-of-fit and correlation coefficients. Note that error bars are within the symbol.

zole and HPβCD (Peeters et al., 2002). When 0.01 or 0.1N HCl was used as the complexation medium, the relative order of the cyclodextrin with regard to solubilization switched such that the SBEβCD become the better solubilizer. This, along with the effects seen at pH 4, is consistent with the work of Zia et al. (2001) wherein ionization of bases tended to reduce interactions with HPβCD while association constants with SBEβCD were less effected. Furthermore, increasing ionic strength tended to reduce complexation efficiency between SBEβCD and charged basic drugs due to charge shielding which minimizes the coloumbic attraction. In any case, at 10% w/v cyclodextrin, SBEβCD solubilized approximately 1600 and 5500 μg/mL itraconazole at 0.01 and 0.1N HCl, respectively, while the corresponding values for HPβCD were 253 and ~2500 μg/mL itraconazole, respectively. At pH 2 (0.01N HCl) the isotherms for both HPβCD and SBEβCD were best fit to a quadratic function (Fig. 6). This behavior had been previously reported for HPβCD by Peeters et al. (2002) and Miyake et al. (1999). At pH 1, the isotherm for HPβCD could best be fit to a quadratic function while that for SBEβCD was linear (Fig. 7). The effect of decreasing pH on the stoichiometry of complexes for HPβCD with weak bases has been suggested to be related to changes in structural affinity as a function of protonation (Peeters et al., 2002; Johnson et al., 1994). The SBEβCD data are interesting. As indicated above, the geometry and structure of this highly anionic cyclodextrin argues against the formation of higher order complexes especially given the conformational nature of the itraconazole molecule (Loftsson et al., 2002; Peeters et al., 2002; Zia et al., 2001). Having said that there seems to be a change in isotherm curvature for the SBEβCD–itraconazole interaction in going from pHs 7 and 4, to pH 2 (0.01N HCl) to systems studied in 0.1N HCl. Whether this is related to non-complex features of cyclodextrin solubilization or other effects still needs to be investigated. It is possible that the low pH or higher ionic strength would not only change the drug but also the manner in which the cyclodextrin interacts with the drug. The pK_a of the SBEβCD is at least 2 units lower than the pH of the media at 0.1N HCl but charge shield or other effects might be involved as suggested above.

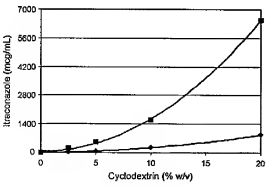


Fig. 6 – Effect of HPβCD (diamonds, blue line) and SBEβCD (squares, pink line) on the solubility of itraconazole in 0.01N HCl ($n=3$). The calculated lines represent fitting of the data to a second-order model which was optimal in this case based on goodness-of-fit and correlation coefficients. For HPβCD, $r^2 > 0.999$ and for SBEβCD, $r^2 > 0.999$. Note that error bars are within the symbol.

3.2. *Solubilization by surfactants*

Surfactants can enhance the solubility of poorly water-soluble lipophiles through micelle formation and other mechanisms (Rangel-Yagui et al., 2005; Torchilin, 2001). Solubilization of a drug by a surfactant is given by the following equation:

$$S_{\text{Total}} = S_0 + \varepsilon(C_{\text{Surfactant}} - \text{CMC})$$

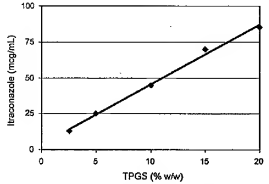


Fig. 8 – Effect of TPGS on the solubility of itraconazole.

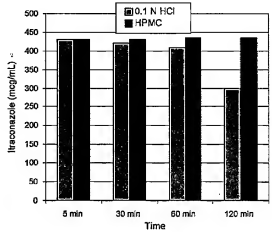


Fig. 9 – Supersaturation data for itraconazole in either 300 mL 0.1N HCl or 300 mL 0.1N HCl with 500 mg HPMC. Equilibrium solubility of itraconazole at 0.1N HCl is 7 μg/mL.

where S_{Total} is the total concentration of drug solubilized, S_0 is the solubility of drug in the absence of the solubilizer, $C_{Surfactant}$ is the concentration of surfactant, CMC is the critical micellar concentration and κ is defined as:

$$\kappa = \frac{S_{Micelle}}{C_{Micelle}}$$

where $S_{Micelle}$ is the drug concentration within the micelle and $C_{Micelle}$ is the molar concentration of surfactant (Loftsson et al., 2002; Yalkowsky, 1999). If the CMC is significantly lower than the concentration of surfactant used, the solubility relationship simplifies to:

$$S_{Total} = S_0 + \kappa C_{Surfactant}$$

The interaction of itraconazole with TPGS is illustrated in Fig. 8. The CMC of TPGS is 0.02 w/w% meaning that the concentrations used in this study were all well above the CMC (Constantinides et al., 2006). The data indicate a linear relationship between the solubilizer and drug ($r^2 = 0.995$) with equilibrium solubilities of 13 μg/mL at 2.5 w/w% and 85 μg/mL at 20 w/w% TPGS. The κ value is 8.95×10^{-4}.

3.3. Supersaturation assays

To evaluate supersaturation, an assessment was completed for itraconazole in the presence and absence of HPMC. The equilibrium solubility of itraconazole at room temperature in 0.1N HCl is 6 and <1 μg/mL in 0.01N HCl. In a first series of experiments, the hypothesis that HPMC stabilize supersaturated solutions of itraconazole was assessed. For this purpose, 300 mL of 0.1N HCl was used as the dissolution medium with or without added HPMC. Using the co-solvent approach, a solution of 50 mg/mL itraconazole in DMF was added to the media to generate a supersaturated solution of itraconazole at approximately 400 μg/mL (66-fold increase over the equilibrium solubility) (Raghavan et al., 2001, 2003; Davis and Hadgraft, 1991). These levels were not physically stable and the drug concentration were reduced by 25% at the 120 min time point (Fig. 9). The total volume of DMF added to the dissolution vessel was <1%. When HPMC is added, the supersaturation levels are maintained through 120 min suggesting that this excipient improves the physical stability of the formed supersaturated solution. Having said that there was no effect on the level of supersaturation. HPMC and PVP have been shown to act as a solubilizer for various compounds including acetazolamide, prazepam and sulfamethoxazole via polymer–drug complexation (Loftsson et al., 1996). In this instance, solubilization of itraconazole by these polymers is negligible.

While this approach was useful in assessing the effect of excipients on supersaturation, it was cumbersome, required large volumes of dissolution medium and thus did not lend itself to screening. To address these issues, a downsized supersaturation test was developed to assess the effect of various excipient on both the extent of supersaturation and the stability of the formed supersaturated solutions (Vandecruys et al., 2006, 2007). The approach was again based on the co-solvent/solvent quench method but the assay was completed using 10 mL of a 0.01N HCl dissolution medium. This medium was selected based on both physiological relevance (it is similar to SGF without pepsin) and breadth of application since this was developed as a general screening tool. In the assay, three surfactants (Tween20, RH40, TPGS), two hydrophilic cyclodextrins (HPβCD and SBEβCD) and PEG4000 were used. Data from a screening assay are presented in Fig. 10. In the media without additives, addition of itraconazole in DMF generated a supersaturated phase with concentration of 17 μg/mL. There was little if any effect of PEG4000 with supersaturation values and durations being similar to the blank. Addition of surfactants to the dissolution media allowed higher levels of itraconazole to be generate such that 400, 620 and 1450 μg/mL were obtained in the presence of Tween20, Cremophor RH40 and TPGS, respectively. These values are supersaturated relative to the equilibrium values for itraconazole in media containing these surfactants. For TPGS, for example, 2.5% w/w of the excipient generates solubility for itraconazole of 13 μg/mL. The physical stability of kinetic systems was limited with the original supersaturation concentration in the Tween20 system reduced by 83% at 30 min and >95% by 120 min. Cremophor gave similar

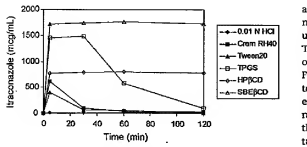


Fig. 10 – Supersaturation data for itraconazole in the presence of various excipients. Equilibrium solubility at the same excipient concentration (2.5% w/v) for TPGS was 13 μg/mL, HPβCD was 21 μg/mL and SBEβCD was 219 μg/mL.

results. TPGS was associated with a somewhat more robust profile with maintenance of supersaturation through 30 min, a 60% decline in concentration by 60 min but a greater than 90% decline by 120 min.

The cyclodextrins tested gave a different profile. The addition of 2.5% w/v HPβCD into the dissolution media resulted in a measured concentration of 770 μg/mL which represented an increase above both the sample without the excipient (16 μg/mL) as well as the solubility determined at equilibrium in phase-solubility analysis (21 μg/mL). Note that the organic solvent (DMF) at the concentrations present in the samples (3.3% w/v in the worst case) did not affect the solubility of itraconazole (data not shown). Furthermore, the concentration of drug in the system was maintained throughout the time course of the experiment. The effect of the SBEβCD was even greater with itraconazole concentrations increased to 1700 μg/mL and good stability over the 120 min experiment. Again these levels were in excess of those generated in the absence of an excipient or at equilibrium in 0.01N HCl containing 2.5 w/v % SBEβCD (205 μg/mL). Interestingly, even though SBEβCD is a better complexing agent for itraconazole in 0.01N HCl, the extent of supersaturation is greater for the HPβCD system (36 vs. 8-fold). This may suggest that mechanisms other than simple complex formation are responsible for the stabilizing effects which may include inhibition of nucleation and crystal growth. Based on these screening experiments, more detailed assessments were completed on the supersaturation effects of TPGS, HPβCD and SBEβCD as presented in Table 1. These data, completed in triplicate, give an indication of the robustness of the technique in that a comparison of Fig. 10 and Table 1 suggest reasonable concordance. The patterns generated were similar with regard to supersaturation effects. In addition, the stability of the formed supersaturated systems was followed over a 24 h time course rather than 120 min. The itraconazole concentrations in media containing TPGS were stable through 60 min (rather than 30 min in the screening experiment) however the concentration in solution at 120 min were only 6% of that at 60 min. Again, the cyclodextrins better support supersaturation with minimal loss through 240 min followed by a decline such that the 24 h sampling point was 8% of the 240 min values in the case of HPβCD and 12.5% in the case of the SBEβCD. Interestingly, the standard deviations associated with supersaturated concentrations of itraconazole increase at the time point(s) just prior to solution "collapse" as seen at 60 min for TPGS, 180 and 240 min for HPβCD and at 240 min for SBEβCD. At 24 h, the values for itraconazole were 13-fold and 40-fold higher in the HPβCD and SBEβCD systems, respectively, when compared to the solvent blank.

4. Discussion

As reviewed by Macie and Grant (1986), the actual process of nucleation is not known with any degree of certainty but a nucleus must form to serve as a center for deposition of solute from the supersaturated system allowing for crystal growth. Crystal growth is believed to take place in three steps including (1) diffusion of the molecule from the bulk media to the solid crystal interface, (2) the adsorbed molecule, through a surface reaction, becomes part of the crystal lattice and the heat of crystallization released and (3), the heat of crystallization is conducted to the bulk medium.

The rate at which nucleation and crystallization takes place is determined by a number of thermodynamic, kinetic and molecular recognition phenomenon (Miller et al., 2007). A solid phase will crystallize out of solution if the chemical potential of the solid phase is less than that of the dissolved phase. In order for crystallization to proceed, supersaturation must

Table 1 – Effect of selected excipients at 2.5% w/v on the concentration ± S.D. (n = 3) and solution physical stability of itraconazole added in a DMF solution from 5 min to 24 h post-addition

Time (min)	Itraconazole (μg/mL)			
	0.01N HCl	TPGS	HPβCD	SBEβCD
5	15.6 ± 0.1	1365 ± 13.5	775.0 ± 6.1	1739.3 ± 13.2
30	15.5 ± 0.3	1373 ± 16.8	778.7 ± 3.2	1731.0 ± 4.4
60	15.1 ± 0.2	1215 ± 114	772.8 ± 2.8	1718.3 ± 23.6
120	15.1 ± 0.2	79.2 ± 3.6	774.3 ± 5.8	1713.0 ± 5.3
180	10.1 ± 1.3	66.2 ± 3.2	757.0 ± 17.8	1683.7 ± 17.0
240	5.8 ± 0.4	61.9 ± 3.2	736.7 ± 15.2	1379.7 ± 373.2
1440	4.3 ± 0.1	45.3 ± 2.9	58.3 ± 2.2	173.7 ± 9.5
Final pH	2.02	2.17	2.10	2.29
DMF% in final solution	0.03%	2.9%	1.5%	3.3%

occur as this is the driving force for nucleation and crystal growth. The rate of nucleation is generally expressed by the following equation:

$$J = N_0 \nu \exp\left(\frac{-16\pi\nu^2\gamma_{12}^3}{3(k_B T)^3(\ln(c/s))^2}\right)$$

where J is the number of nuclei formed per unit time per unit volume, N_0 is the number of molecules of the crystallizing phase per unit volume, ν is the frequency of transport through the nucleus–liquid interface, υ is the molecular volume of the crystallizing solute, γ is the interfacial energy per unit area, k_B is the Boltzmann constant, T is temperature and c/s is the extent of supersaturation. As described by Miller et al. (2007), solubility, interfacial tension and viscosity are the solvent-related features most likely to affect nucleation.

Materials that may inhibit nucleation or crystal growth have been reported (Raghavan et al., 2001, 2003; Gao et al., 2004; Terayama et al., 2004). These materials have several potential actions including altering bulk properties such as surface tension or saturation solubility, changing the adsorption layer at the crystal–medium interface, selectively adsorbing to the crystal interface thereby blocking crystal growth, being adsorbed into growth layers and thereby disrupting growth layers across the surface, adsorbing into surface imperfections causing rough surfaces to become flat and altering the surface energy of the crystal face.

The effect of pharmaceutical polymers such as methyl cellulose (MC), HPMC and PVP has traditionally been ascribed to several of the effects described above (Raghavan et al., 2001). Simonelli et al. (1970) suggested that the inhibitory effect of PVP on the nucleation and growth of sulfathiazole was associated with the polymer forming a porous net around growing crystals such that crystal growth was relegated to the formed pores in the polymeric network. Others have suggested that the polymers adsorb to the crystal thereby inhibiting the addition of molecular units from the bulk solution (Raghavan et al., 2001). Additional mechanisms that have been forwarded include the suggestion that these polymers can solubilize the drug of interest through complexation thereby reducing the degree of supersaturation (Rodriguez-Hornedo and Murphy, 1999; Strickley, 2004). Raghavan et al. (2001, 2003) correlated the hydrogen bond donor and acceptor properties of hydrocortisone acetate, HMPC and PVP to explain the differential inhibitory effect of the polymers on drug nucleation and crystal growth. The working theory developed included the interaction of the polymer in the unstirred water layer separating the growing crystal and the bulk media. Specifically, the polymers can hydrogen bond to growth sites on the crystal thereby blocking growth. In addition, even though these polymer may be rejected at the crystal interface, they may, for a variety of reasons, accumulate in the unstirred water layer and increase resistance to diffusion. These effects were predicted to be higher for those faces of the growing crystal that have a higher hydrogen bonding potential meaning that the habit of the crystals may change along with their growth rates. Yokoi et al. (2005) suggested HPMC and sugar esters (sucrose fatty acid esters) exerted differential effects on the stabilization of aqueous suspensions of cefditoren pivoxil based on the their nature and extend of surface binding. Thus, the interaction of HPMC was such that crystal growth was selectively inhibited where the sugar esters were predicted to inhibit both nucleation and crystal growth.

In the case of hydrophilic cyclodextrins, Xiang and Anderson (2002) found that the generation of supersaturated solutions of a novel anti-cancer agent (Silatecan) was possible by converting a precursor to the lactone at an appropriate pH in a SBEβCD solution. These systems were stable for up to 3 days after preparation and could be lyophilized and successfully reconstituted. Uekama et al. (1992) have also assessed the ability of CD to inhibit crystal growth in drug formulations. In a spray-dried dispersion of nifedipine and HPβCD, crystal growth was minimized such that neither dissolution changes nor attenuation of oral bioavailability in dogs were observed in aged material compared to freshly prepared dispersions. Furthermore, Torres-Labandeira et al. (1991) found that supersaturated solutions of pancratistatin could be prepared in HPβCD solutions. In their preparation procedure, the drug was treated with ammonia after which the aqueous ammoniacal solution was removed by freeze-drying. Reconstitution of the powder allowed for solutions as high as 9 mg/mL to be prepared. While precipitation was observed in these samples, the use of polyethylene containers as well as HPγCD provided for a significantly prolonged latency period prior to precipitation. Iervolino et al. (2000) reported that HPβCD inhibits nucleation of ibuprofen in supersaturated PG-water systems and distinguished this effect from that of HPMC which was effective in preventing crystal growth. Dias et al. (2003) suggested a stabilizing effect of HPβCD–HPMC systems on supersaturated diclofenac solutions. On the other hand, Ma et al. (1996) found that HPβCD accelerated crystal growth of norethindrone acetate in a supersaturated acrylic adhesive.

The suggested mechanism for inhibition of nucleation and crystal growth observed in the case of pharmaceutical polymers may also apply to the chemically modified hydrophilic cyclodextrins examined. Specifically, cyclodextrins solubilize itraconazole by inclusion complex formation with possible contribution from non-complex-based mechanisms. This increases the chemical potential of the drug in solution, increases the apparent saturation solubility and decreases the extent of supersaturation (Iervolino et al., 2000, 2001; Strickley, 2004). As noted, this effect alone does not seem capable of accounting for the effect on the formed supersaturated solution based on the magnitude of the measured changes on apparent solubility. On the other hand, Iervolino et al. (2000) suggested that the interaction of ibuprofen with HPβCD changes the metastable zone as a function of drug–cyclodextrin interaction and solubilization. The cyclodextrins may also interact with the growing crystal in a manner analogous to HMPC with hydrogen bonding to sites associated with crystal growth as well as accumulation in the unstirred water layer resulting in an increase in diffusional resistance secondary to viscosity or even the ability of the cyclodextrin to complex with drug monomers inhibiting efficient mass transfer at the interface. In some cases, the preparation method can promote the formation of supersaturated systems as in cases where ammonia or volatile acids are added to facilitate complexation and then removed via lyophilization (Loftsson et al., 2004c). Pedersen (1997) suggested that supersaturation was more likely when a molecular

complex was assessed compared to freeze-dried or ground materials containing mixture of the drug and cyclodextrin based on the rate of cyclodextrin dissolution. For complexes, the dissolution rate is limited meaning that drug release as a function of cyclodextrin availability provides for supersaturation in contrast to other systems where the rate of cyclodextrin dissolution is larger providing a greater opportunity for solution complexation and an increase in the saturation solubility of the drug.

Loftsson et al. (2007) has also suggested that the manner by which excipient alter the cohesive nature of water may effect induction time and nucleation. To this point, materials that enhance water structure (i.e., kosmotropes) may stabilize supersaturated solution while those reducing water structure (i.e., chaotropes) may have the opposite effect. Cyclodextrin have been suggested to be sugar-like in their effect acting as a kosmotrope at higher concentrations. Finally, certain types of cyclodextrin nanoparticles can be formed using techniques similar to the solvent quench approach used herein (Duchêne et al., 1999). Importantly, these techniques have been applied to amphiphilic cyclodextrin derivatives in which the hydroxyl functions are appended with alkyl ethers, esters or amides would seem less relevant to hydrophilic cyclodextrins such as HPβCD and SBEβCD. Having said that, the aggregation of hydrophilic cyclodextrins has been suggested in aqueous systems (Brewster and Loftsson, 2007).

5. Conclusions

Cyclodextrins have long been valued for their pharmaceutical properties which enable the formulation of a number of biopharmaceutically challenging drugs and drug candidates. The basis for these effects were generally attributed to the ability of cyclodextrins to form dynamic inclusion complexes in solution. Recent data suggest that some of the solubilizing potential of the cyclodextrins may be related to non-inclusion complex related phenomena such as cyclodextrin aggregation or surfactant-like properties inherent in these systems. A third possible action for these useful excipients may be their ability to stabilize formed supersaturated solutions. Such effects may be very useful in the preparation of dosage forms that are designed to operate under carrier-controlled dissolution (i.e., solid dispersions) and give rise to supersaturation and improved oral bioavailability.

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